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Prognostic value of somatostatin receptor subtype 2 expression in colorectal cancer

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Abstract

The clinical relevance of the somatostatin receptor subtype 2 (sst2) is well defined in neuroendocrine tumors but it is still a matter of debate whether its expression may have a role also in other tumors not arising from the neuroectoderm. We investigated the prognostic value of the expression levels of sst2 mRNA in a consistent group of patients affected by colorectal cancer. Survival analysis of cancer-related death showed that patients with a high sst2 mRNA expression had an unfavourable outcome ($p=0.037$) and a significantly shorter disease-free survival ($p=0.008$). Surprisingly, our findings suggest that *sst2* gene overexpression is a feature of colorectal tumors that have a negative outlook; in addition, it may allow additional insight into conventional therapeutic approaches for more aggressive tumors, whose prognosis needs to be improved.

Keywords: sst2 mRNA; Real time RT-PCR; Survival

1. Introduction

Somatostatin (SS) is a widely distributed, multifunctional inhibitory peptide hormone which is involved in multiple cellular activities. In particular, SS regulates cell secretion and proliferation through a family of specific G-protein coupled receptors (ssts) [1]. The role of one of these receptors in particular, namely, type 2 (sst2), which strongly mediates the antiproliferative action of SS and shows high affinity for the currently available SS analogs, has been clearly established for both endocrine and neuroendocrine tumors [2]. Indeed the presence of sst2 provides a strong basis for diagnosis and treatment of the majority of endocrine and neuroendocrine tumors expressing the receptor [3]. Also, we reported that the expression of sst2 was positively related to patient outcome in the childhood tumor neuroblastoma, therefore giving relevant insights in terms of patients overall and disease-free survival [4].

Many recent studies showing that common solid tumors, such as colorectal and breast cancer, often express these receptors, have led to growing interest in the clinical utility of ssts as prognostic and therapeutic targets for these tumors also. With respect to colon cancer, therapy with SS analogs has been generally disappointing in terms of both survival and disease stabilization in the majority of the reported trials performed randomly on patients affected by this tumor [5–7]. In only one study a significant advantage in terms of survival has been reported [8]. We believed that these findings could be explained by several factors, such as the

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different dose of SS analogs given to patients in different trials, the inappropriate selection of patients (highly disseminated disease), and the lack of investigation regarding the presence of ssts in the tumors [9]. Indeed, in the aforementioned trials the ssts status of patients has not been elucidated before initiation of therapy, further complicating the interpretation of the findings obtained so far [10]. Regarding this aspect, the few studies that were performed to characterize the pattern of expression of different ssts subtypes in colon cancer have provided controversial results [11–14], which in our opinion could also be explained by the methods employed. Furthermore, our data on neuroblastoma clearly showed that only a quantitative determination of *sst2* gene expression had a significant prognostic value [4].

In our previous study we evaluated sst2 mRNA expression by quantitative RT-PCR in sporadic colorectal carcinomas and in their paired adjacent unaffected tissues [15]. Since long-term follow-up has now reached a significant period, we investigated whether the quantitative determination of sst2 may have had a prognostic relevance in the same group of patients.

2. Materials and methods

2.1. Patients and samples

Tissues were obtained from 96 patients with sporadic colorectal carcinoma, scheduled for elective resection. Informed consent was previously obtained from all patients. For all patients at least one sample of both neoplastic and normal tissue (taken 10 cm apart from the neoplasm) were obtained. Samples were immediately snap frozen and stored in liquid nitrogen. Tumor was localized in the right colon in 33 patients, in the left colon (12 in the descending, and 21 in the sigmoid colon) in 32 patients, and in the rectal portion in the remaining 31 patients. Histological examination was performed routinely in all cases. An adequate number of sections were sampled from each tumor. Tumor histotype and grade of differentiation were defined according to the World Health Organization criteria [16]. The pattern of cancer growth was assessed as expanding (when the tumor border was clearly demarcated) and as infiltrating (when cancer cells spread into the surrounding tissues without a distinct border) [17]. All cases were staged according to the original Dukes' system. According to the histopathological grading, 5 tumors were G1, 61 were G2, 8 were G3 and 16 were colloid. Six were in situ tumors.

Total RNA was extracted from each sample with RNeasy Kit (Quiagen S.p.A., Milan, Italy). Since *sst2* is an intronless gene, each RNA sample was first submitted to a conventional PCR with the same primers and cycling for sst2, but without reverse transcription, to exclude the presence of residual genomic DNA in the extracted specimens. Samples with residual DNA were treated with DNAse, till the disappearance of any DNA trace.

2.2. Quantitative evaluation of sst2 mRNA expression

The primers and probe for sst2 mRNA quantification to use with the ABI Prism 7700 Sequence Detection System were described elsewhere [18]. Four hundred nanograms of total RNA were reverse-transcribed according to recommended protocol. The PCR mixture contained primers (200 mM each) and 200 nM of the Taqman probe, in a final volume of 25 microl. Amplification and detection were performed with the ABI Prism 7700 Sequence Detection System with the following profile: one step at 50 °C for 2 min, one step at 95 °C for 10 min, and 40 cycles at 95 °C for 30 s and 60 °C for 1 min. The amount of product was measured by interpolation from a standard curve with RNA extracted from neuroblastoma cell line CHP404, which over-expresses sst2 mRNA. One microgram of CHP404 RNA was reverse transcribed and cDNA is then serially diluted to obtain 5 standard solutions to be used in the PCR reaction to generate the reference curve [18].

2.3. Statistical analysis

Statistical analysis was carried out using the SPSS software package (SPSS INC, Chicago, IL). For analysis of follow-up data, life table curves were calculated using Kaplan–Meier method and survival distribution were compared by log-rank statistics. The primary end point was cancer-related survival, as measured from the date of surgery to the time of last follow-up or cancer-related death. The joint effects with already recognized prognostically relevant variables were examined via Cox proportional hazard analysis. Pattern of growth and Duke's stage were entered stepwise forward into the model to test these covariables for possible joint effects with high/low levels of sst2 expression. Differences were considered statistically significant with $p < 0.05$.

3. Results

Our results showed that sst2 was variably expressed in all colon cancers investigated and, on average, tumor samples expressed a lower amount of sst2 mRNA than the unaffected samples [15]. Taking into account the variability due to the assay procedure, we assumed that sst2 mRNA was over-expressed in the tumor tissue when its concentration was at least twice the value in the respective adjacent unaffected tissue. Survival analysis of cancer-related death, obtained by comparing tumors over-expressing sst2 mRNA (cancer >2-fold adjacent unaffected tissue) vs tumors with a low sst2 mRNA expression (cancer ≤2-fold adjacent unaffected tissue), showed a significant correlation of high expression of sst2 mRNA with unfavourable outcome (log-

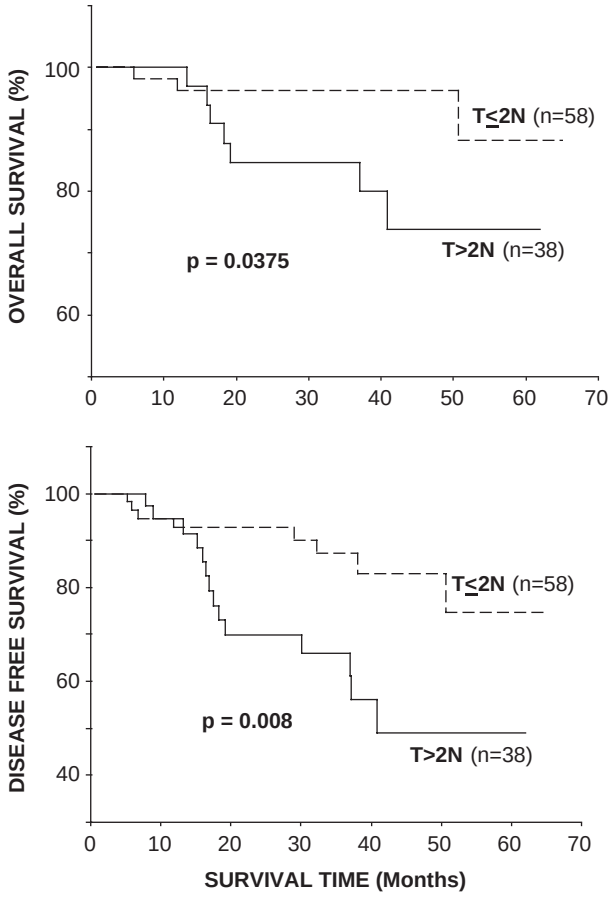

Fig. 1. Cumulative cancer-related survival in patients over-expressing sst2 mRNA [cancer mRNA expression >2-fold than in adjacent unaffected tissue ($T>2$ N)] vs tumors with a low sst2 mRNA expression [cancer mRNA expression ≤2-fold than in adjacent unaffected tissue ($T\leq 2$ N)], showed a significant correlation between high expression of sst2 mRNA and unfavorable outcome (upper panel, log-rank test, $p=0.0375$) and disease-free survival (lower panel, $p=0.008$). Analysis of distributions were calculated using Kaplan–Meier method and compared by log-rank test.

rank test, $p=0.037$) and shorter disease-free survival ($p=0.008$) (see Fig. 1). With respect to some well-established prognostic factors (stage according to Duke classification, and growth pattern), cancer sst2 overexpression correlated significantly with overall survival in univariate Cox regression analysis. In multivariate analysis, sst2 mRNA over-expression was shown to be an independent prognostic parameter for overall survival ($p=0.027$) (see Table 1).

4. Discussion

As already mentioned, according to the data obtained so far there is clear evidence that SS analogs are highly effective in the symptomatic management of patients with neuroendocrine tumors [19,20]. This still has to be elucidated for malignancies not arising from the neuroectoderm, in which the presence of ssts has though been demonstrated.

Also, we showed that in neuroblastoma the quantitative determination of *sst2* gene expression could provide relevant prognostic information, independently from the other well known prognostic markers [4].

In our previous study [15], beside a variable presence of sst2 in all colon tumors investigated, we did not observe any statistically significant relationship between sst2 expression and any of the parameters examined such as localization, grading and stage of the disease. However loss of sst2 seemed to be a relevant event in patients with high preoperative concentration of carcinoembryonic antigen, a poor prognostic indicator for colorectal carcinoma [15].

Our current findings on the same group of patients do not confirm this previous observation; surprisingly, they show a significant correlation between high expression of sst2 mRNA, unfavourable outcome and shorter disease-free survival.

Indeed, these findings are in contrast not only with the aforementioned data in neuroblastoma, but also with results we recently obtained in a prospective study on a large group of patients affected by breast cancer [21]. In the latter we found an upregulation of sst2 mRNA expression in those breast tumors that on the basis of conventional predictive parameters are expected to have a better prognosis.

In our opinion, the clinical and pathological relevance of the presence of ssts in human primary non-neuroendocrine tumors, such as colorectal cancer, remains unclear. Moreover, the clinical studies with SS analogs performed up to now in patients affected by colon cancer do not justify the routine use of such treatment in the management of this malignancy. Indeed clinical evidence of the antiproliferative effect of SS analogs is restricted to acromegalics and to some patients with gastrointestinal neuroendocrine tumors, i.e. to tumors with a very high sst2 density [22].

Beside their prognostic relevance, our results may suggest the opportunity of adding SS analogs to conventional treatment modalities in a specific subset of patients affected by colon cancer. Tumors with a relatively higher sst2 expression may represent the ideal target for a treatment based on SS analogs, particularly in conjunction with radio-

Table 1
Univariate and multivariate cox regression analysis for 96 patients with resected colon carcinoma

Variable	Univariate analysis	Multivariate analysis		
	p	*p*	Relative Risk Exp (B)	Exp (B) 95% Cl
Pattern of growth Infiltrating vs pushing	0.048	0.114	0.179	0.021–1.511
Dukes' Stage A+B vs D+C	0.022	0.283	2.123	0.537–8.395
sst2 mRNA ratio[a] >2 vs ≤2	0.037	0.027	5.875	1.225–28.174

[a] sst2 mRNA was over-expressed in colon cancer when its concentration was at least twice than in the respective adjacent not affected tissue.

emitting molecules or chemotherapeutic agents for receptor-mediated therapy [23]. This is particularly important for tumors which turn out to be more aggressive, and for whom conventional therapies need to be improved.

Finally, it remains to be elucidated if the variable expression of sst2 in colorectal cancer might have a relevance in exploiting the therapeutic effects of SS analogs, and therefore if investigation of ssts tumor status before initiation of therapy could represent a tool for predicting the efficacy of cold or radio-labelled SS analogs.

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